

AUTOLOGOUS CULTURED FIBROBLASTS AS CELLULAR THERAPY IN PLASTIC SURGERY

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Autologous cultured fibroblasts (Isolagen; Isolagen Technologies, Paramus, NJ) are a living cellular therapy for the correction of rhytids and scars. The treatment of the dermis with cultured autologous fibroblasts can restore the population of fibroblasts that are reduced as the result of photodamage, aging, and scarring. Isolagen represents a living dynamic protein repair system that creates an early moderate fill with a continuing gradual correction for a period of 12 to 24 months after treatment. The correction has been long lasting and has not shown a dissipation of the correction throughout the 4½ years of the authors' investigations (Fig. 1). The use of autologous cultured fibroblasts (Isolagen) as an injectable system to repair dermal and mild subcutaneous defects such as rhytids, acne, and scars is a new treatment modality available to plastic, cosmetic, and reconstructive surgeons. Isolagen represents the first attempt at cellular therapy in the field of plastic surgery and dermatology. The potential beneficial effects of replenishing the dermis and subcutaneous tissues with autologous fibroblasts have been investigated since 1995 by the lead author.

The commonly used soft tissue fillers should be divided into either dermal or sub-

cutaneous fillers. All these fillers are nonliving materials that are either protein based or synthetic. The approved dermal fillers, such as bovine collagen and cross-linked human collagen, are acellular and resorb within 3 to 6 months.^{4, 14} Purified bovine collagen is available as Zyderm I, Zyderm II, and Zyplast (McGhan Medical, Santa Barbara, CA). In the authors' experience substances usually dissolve within 3 months. Furthermore, 1% to 6% of patients receiving bovine collagen injections develop a localized hypersensitivity reaction.^{5, 12} Granulomatous foreign body reactions have occurred and reactions that have caused multiple nodules, hypopigmentation, and prolonged induration also have been associated with the use of processed collagen.^{2, 3, 11} The synthetic dermal fillers, such as silicone, Artecoll (Rofil Medical International B.V., Breda, The Netherlands), and hyaluronan (Hylaform Gel; Biomatrix, Inc., Ridgefield, NJ), have not been approved for use in the United States. The subcutaneous fillers, such as AlloDerm (Life Cell Corporation, The Woodlands, TX) and SoftForm (Kinamed Incorporated, Newbury Park, CA), are used for the treatment of subcutaneous depressions and should not be confused with the treatment of dermal deficiencies. As subcutaneous

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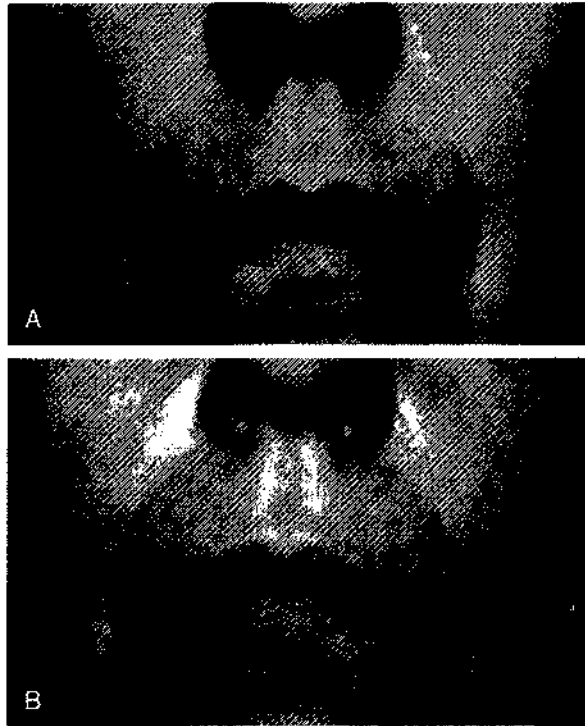


Figure 1. Before (A) and after (B) autologous cultured fibroblasts (Isolagen) injection to perioral rhytids and vermilion.

fillers, these materials require surgical insertion, which carries risk of infection, reabsorption, malposition, palpability, and rejection. Because these materials are for subcutaneous defects, they do not treat acne scars and fine dermal rhytids. They are most appropriate for laugh lines and lips.

To best assess the most appropriate treat-

ment modality from the aforementioned list of materials and treatments, one must make a diagnosis as to the cause and components of the rhytid, fold, scar, or wrinkle that is to be treated. The causative factors of skin irregularities can be related to epidermal, dermal, dermal subcutaneous, and dynamic causes. A diagnosis should be made as to

Table 1. TREATMENT OF CUTANEOUS CONTOUR IRREGULARITIES

Cause of Cutaneous Irregularities	Treatment
Poor qualities of epidermis	Glycolic acid, ascorbic acid, TCA peels Microdermabrasion
Dermal depressions (rhytids)	Superficial Er:YAG lasers Isolagen alone or with deep peels CO ₂ , Er:YAG, Nd:YAG (1032 nm), and Q-switched Nd:YAG (1064 nm) lasers
Subcutaneous defects (i.e., laugh lines, marionette lines)	Injectable collagen products Injectable collagen plus Isolagen Isolagen for mild to moderate defects Fat injections Cadaver dermis
Dynamic rhytids	PTFE Botox Surgery
Scars (i.e., acne, traumatic)	Botox plus fillers or Isolagen Isolagen Isolagen plus use of lasers or peels

which component or components are creating the skin irregularity that the clinician wishes to treat. Table 1 depicts the different treatment modalities for the different causes of skin irregularity. There may be several contributing factors to a skin irregularity and multiple modalities might give the best results.

Cultured autologous fibroblasts should be mutually complimentary to the procedures and treatments listed in the table. Living autologous fibroblasts have the potential to produce collagen, especially when they exist in

large intradermal quantities. The authors' histologic results have shown a significant increase in the thickness of dermis that has been present for over 3 years (Fig. 2). The work of Kuo has shown that collagen production continues after laser therapy.⁸ Goldberg's work has also shown that collagen production can be stimulated by the use of the Q-switched Nd:YAG laser at 1064 nm.⁷ Other studies also have shown rhytid reduction and collagen productions after CO₂ laser treatments.^{6, 10} Moy's studies on glycolic acids

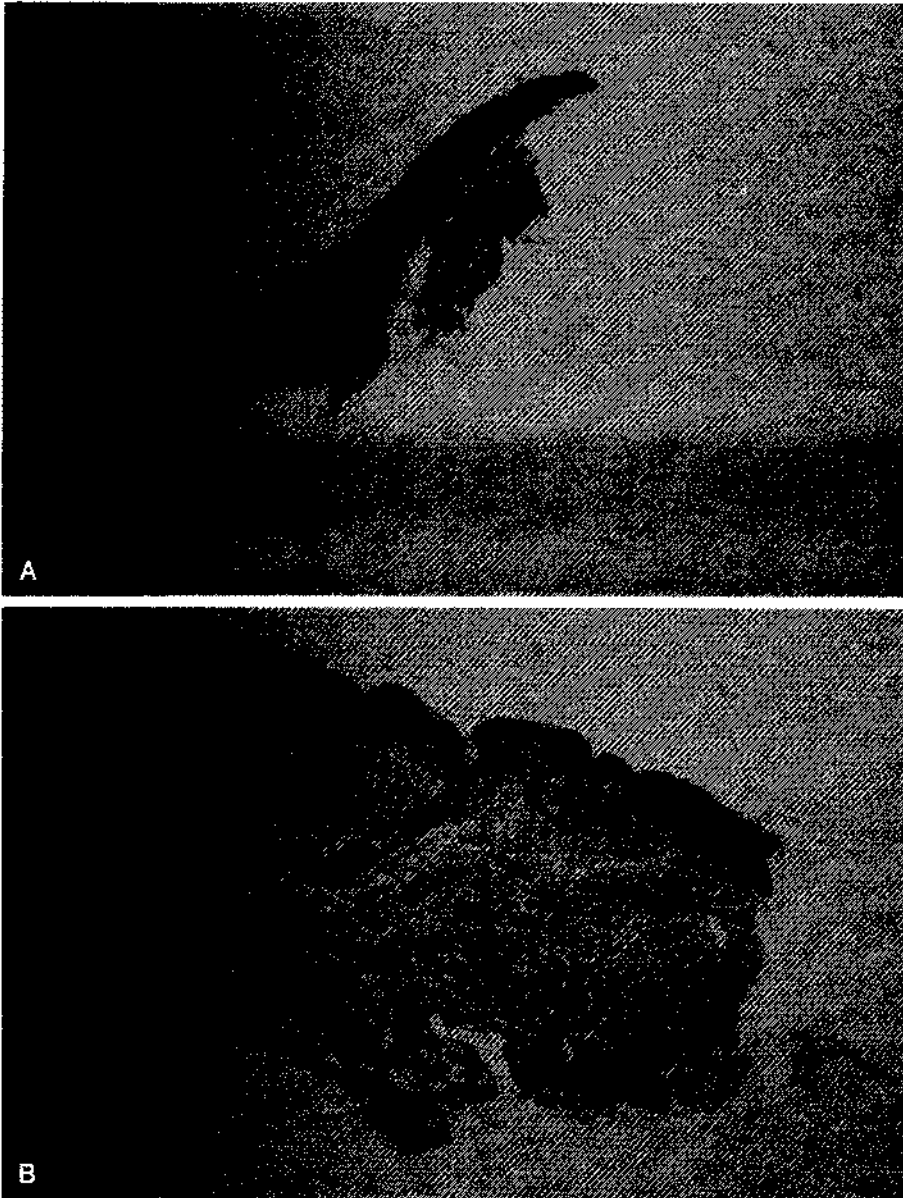


Figure 2. A, Dermal thickness (7 mm) of nontreated area. B, Dermal thickness (2.7 mm) of area 3 years after treatment.

have shown that fibroblasts can be stimulated to produce more collagen in the *in vitro* and *in vivo* environment.⁹ An ideal environment to treat dermal skin conditions is one that is rich in autologous fibroblasts. This synergy can produce dramatic results in combination therapy with lasers and processed collagen, which the authors have seen in the 4½ years of their study.

METHODOLOGY

Before the Isolagen process begins, pretreatment photographs and a specialized informed consent are obtained. The process starts with a 3-mm retroauricular punch biopsy. The surgical specimen is placed in a special transport media tube on ice and in a thermos. The donor site is closed with one buried absorbable suture and the specimen is shipped by overnight mail to the laboratory where the cells are expanded by proprietary tissue culture techniques. Six weeks following the biopsy, the patients are given a test injection that is performed at the wrist level with a 30-gauge needle. Eight weeks following the biopsy, a 1.2-mL sterile vial containing Isolagen is shipped back to the treating physician on ice and in a thermos. The therapeutic injection must be administered within 24 hours of shipping to ensure a 95% viability of the cultured fibroblasts. At 48 hours after shipping, the viability drops to 85%, and at 72 hours the viability is only 65%. Ice is administered to the injection site pre- and postinjection. The treatment areas are outlined with a betadine solution. The Isolagen is injected with a 30-gauge needle to the superficial, middle, and deep dermis. The Isolagen also was infiltrated into the dermal subcutaneous junction and through all levels of a scar. Multiple passes of the 30-gauge needle were used to insert the Isolagen and create pockets within the dermis for the installation of the Isolagen material. The Isolagen also has been used as an adjunct to the subcision technique; it should be injected into the area for treat-

ment at 14 to 21 days following the subcision. Isolagen also has been used as an adjunct to botulinum toxin, and to prolong the results of bovine collagen. All patients were advised to discontinue the use of oral or topical vitamin E and corticosteroids, to take 1500 mg of ascorbic acid orally, and to use either a topical glycolic acid system or an ascorbic acid topical cream. Any patients with a history of autoimmune diseases, disseminated carcinomas, or transplantation, or those patients taking corticosteroids for any reasons, are contraindicated for treatment. Any patients with chronic diseases or skin conditions, such as psoriasis or vitiligo, also are eliminated from the treatment.

CLINICAL EXPERIENCE

Cultured autologous fibroblasts (Isolagen) have been used clinically in the United States and Europe. Over 1250 patients have been treated in the United States, and a total of 4800 injections have been administered to these patients. The major study centers have been at the University of California, Los Angeles (UCLA) Medical Center, the Chernoff Laser Center of Indianapolis, the University of Medicine and Dentistry of New Jersey, and the Hackensack Medical Center (where the lead author has treated 94 patients since 1995). The UCLA Medical Center has treated 40 patients in their clinical study, which evaluated the subjective results determined by two clinicians and the patient and the laser profilometry that provided significant objective data.¹³ In a study of 104 patients at the Chernoff Laser Center, Dr Gregory Chernoff evaluated Isolagen with laser profilometry and the effect of Isolagen pretreatment before 1320-nanometer Nd:YAG laser therapy. Dr Chernoff performed subjective analysis and objective evaluation with laser profilometry and histology.

The study conducted at Hackensack University Medical Center and the University of Medicine and Dentistry of New Jersey began

Table 2. UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY STUDY

	Number of Patients	Short-term Follow-up (12 Mos)	Long-term Follow-up (36-48 Mos)
Patient satisfaction %	94	92%	70%
Grading of correction	56	7.8	6.46

Table 3. CHERNOFF STUDY USING ISOLAGEN AND COOL-TOUCH Nd:YAG LASER

	Average Time to Patient Satisfaction	Number of Patients
Cool-Touch Nd:YAG Laser and Isolagen (Isolagen Technologies, Paramus, NJ)	3.6 mos	20 Females
Control group	4 mos	5 Males
	6.5 mos	20 Females
	7 mos	5 Males

Courtesy of Gregory Chernoff, MD, Indianapolis, IN

in March 1995 and has continued through the present time. The patients were asked to grade their perceived degree of correction on a scale from 0 to 10, with 10 being total and complete correction. The patients also were

questioned about their degree of satisfaction with results, and finally about their perception of continuing noticeable improvements in the treated areas. The average patient grading response with regard to the degree of correction was 7.8, and 92% of the patients reported that they were pleased with the results of the overall program. Of the patients reported, 78% perceived an ongoing and continuing improvement of the treated area for periods up to 24 months (Table 2). There were no reports of infection, rejection, granuloma formation, keloid formation, or overcorrection of the defects in that study.¹

The UCLA study of Watson et al¹³ revealed several significant findings. The patients' perception of the extent of the Isolagen correction directly correlated with the objective improvement that was documented by laser profilometry (Fig. 3). The UCLA study also documented the continuing clinical improvement and patient satisfaction by the patients'

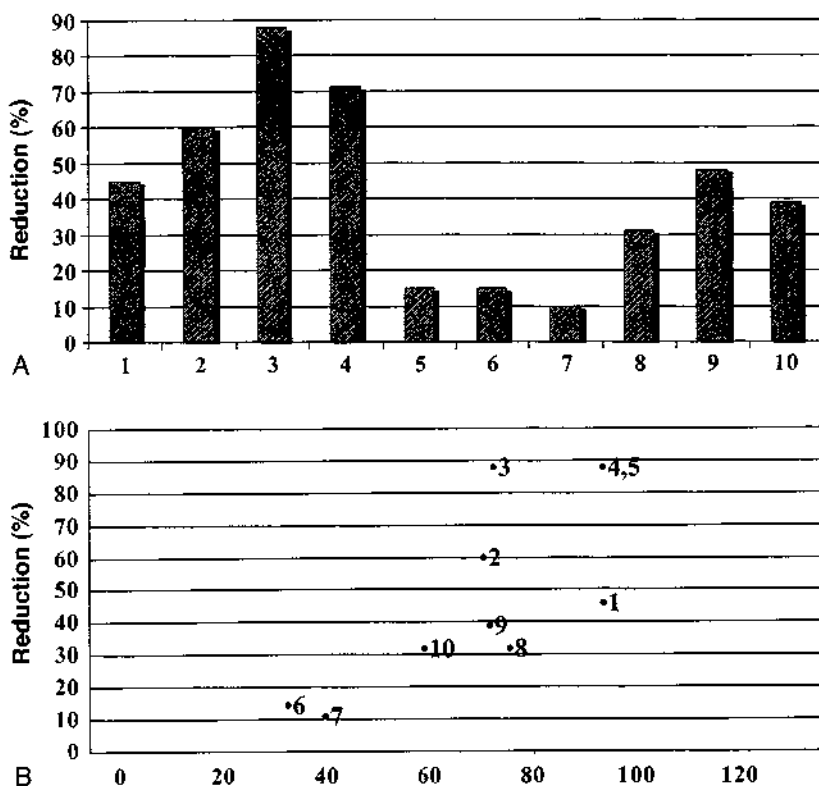


Figure 3. A, Optical profilometry. B, Correlation of profilometry data to subjective improvement scores. Percent reduction of shadow areas by laser profilometry and subjective improvement. (Adapted from Watson D, Keller GS, Lacombe V, et al: Autologous fibroblasts for treatment of facial rhytids and dermal depressions. Archives of Facial and Plastic Surgery 1:165-170, 1999; with permission.)

Table 4. CHERNOFF STUDY ISOLAGEN INJECTIONS

Treatment Areas	Static Rhytids	Acne/Pox Scars	Linear Scars	Lips	Nasolabial Folds	Melolabial Folds	Striae	Nonhealing Laser Wounds
Female (90)	20	30	15	40	14	6	30	10
Male (14)	10	14	5					

Courtesy of Gregory Chernoff, MD, Indianapolis, IN

responses and the two clinical observers. The UCLA study showed a significant degree of improvement at 3 months postinjection, but even a greater degree of correction after 6 months (Fig. 4). The correction was documented by laser profilometry. The laser profilometry study correlated with the clinical observer and patient satisfaction grades.

The summary of the results of the UCLA

study indicated that 9 out of 10 patients reported a 60% to 100% improvement, and laser profilometry demonstrated a 10% to 85% reduction in the scar or rhytids. The histologic studies demonstrated an increased thickness of the dermis with an increased population of fibroblasts and no signs of inflammation (Fig. 5).

The Chernoff study evaluated 104 patients

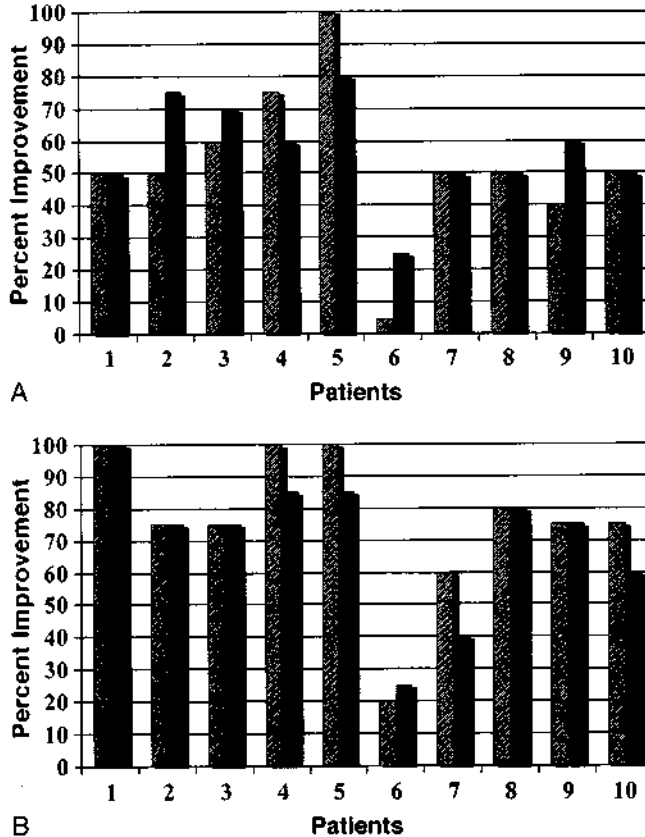


Figure 4. Subject improvement scores. Three months (A) and six months (B) after autologous cultured fibroblasts (Isolagen) injection. Light bar = patient score; dark bar = doctor score. (Adapted from Watson D, Keller GS, Lacombe V, et al: Autologous fibroblasts for treatment of facial rhytids and dermal depressions. Archives of Facial and Plastic Surgery 1:165-170, 1999; with permission.)

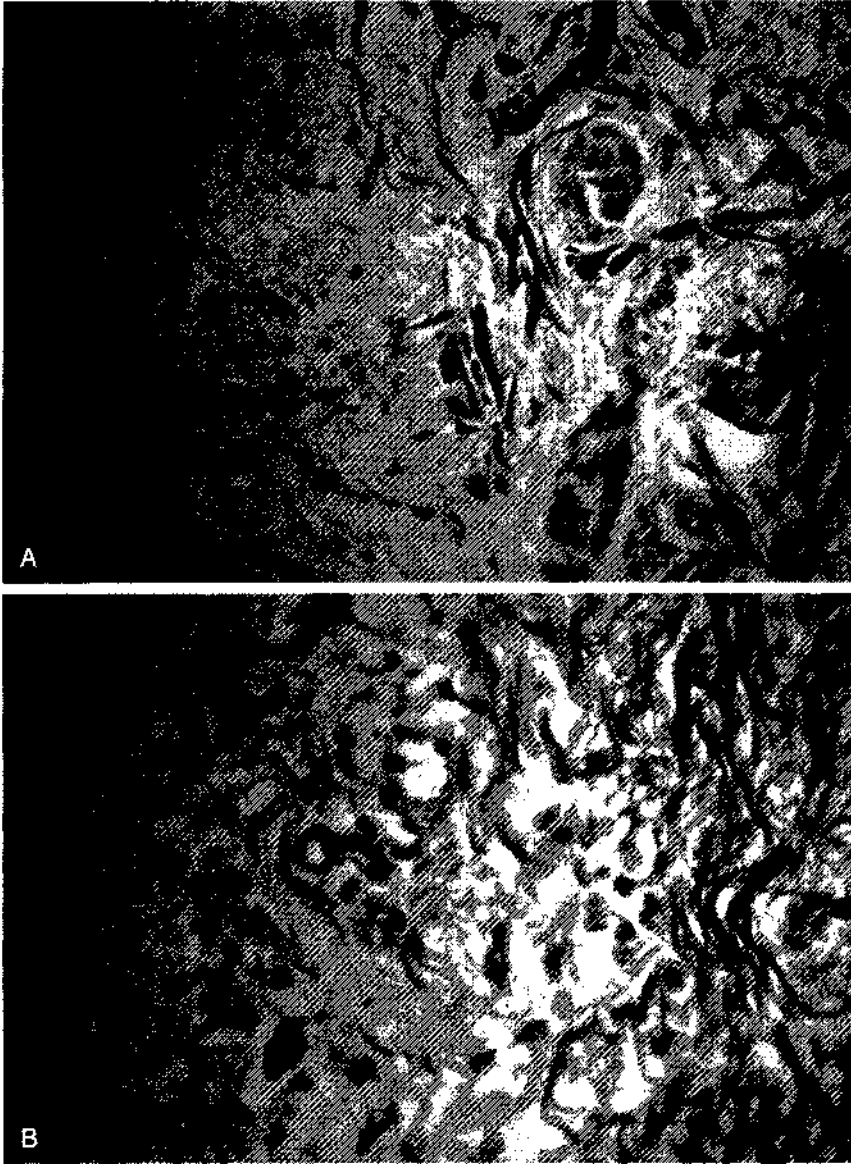


Figure 5. Before (A) and 6 months after (B) autologous cultured fibroblasts (Isolagen) injection. Note increased collagen and fibroblasts with no inflammation after treatment. (Courtesy of Gregory Chernoff, MD, Indianapolis, IN.)

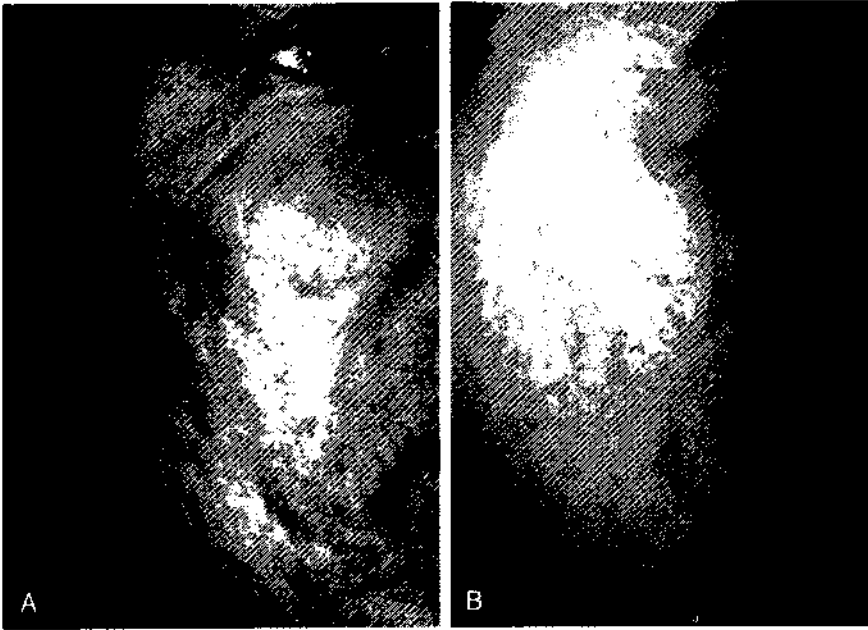


Figure 6. Acne scars before (A) and 6 months after (B) autologous cultured fibroblasts (Isolagen) injection. (Courtesy of Gregory Chernoff, MD, Indianapolis, IN.)

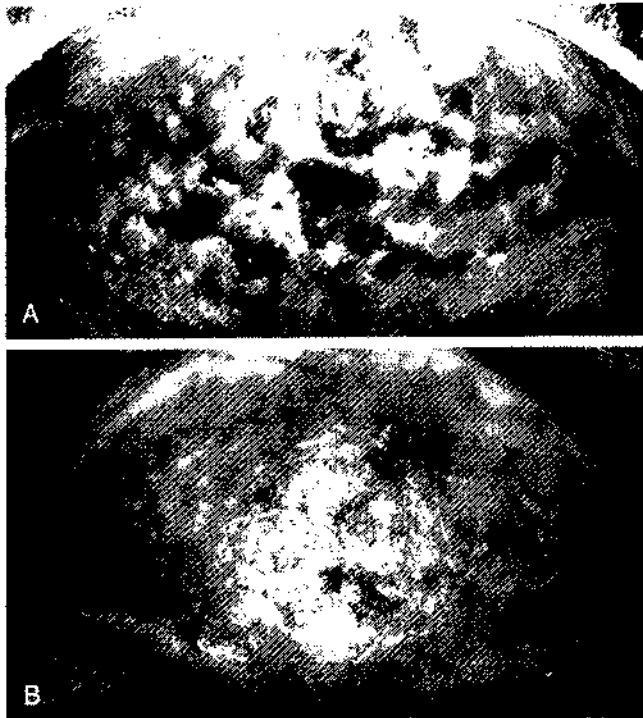


Figure 7. Nonhealing wound, 6 months after CO₂ laser resurfacing (A) and 6 weeks after autologous cultured fibroblasts (Isolagen) injection (B).



Figure 8. Normal structure of neocollagenesis after autologous cultured fibroblasts (Isolagen) injection. (Original magnification $\times 2100$). (Courtesy of Gregory Chernoff, MD, Indianapolis, IN.)

(G. Chernoff, MD, unpublished data, 1997–1998). The patients were treated for rhytids and acne scars. The study was divided into a group that had Isolagen used alone and a group that had Isolagen pretreatment, which then was followed up with the Nd:YAG Cool-Touch Laser at 1320 nm. Chernoff had a third limb of this study in which he used the Isolagen to treat nonhealing laser burn wounds. The Chernoff study ran for 12 months and demonstrated a significant improvement by laser profilometry in the Isolagen treated group, with the most dramatic results being in the younger age group and for acne scars (Fig. 6; Table 3). Dr Chernoff also showed a significant improvement in the results of the Cool-Touch laser when the dermis had been pretreated with Isolagen (Table 4). Finally, Dr Chernoff demonstrated dramatic healing in

patients who had suffered full thickness facial skin loss following laser resurfacing. This group of patients had nonhealing facial wounds for 3 to 9 months and became totally epithelialized within 6 weeks of Isolagen therapy (Fig. 7). Dr Chernoff's studies also were backed up by histologic documentation of increased collagen formation, increased numbers of fibroblasts in the dermis, and no inflammatory response in the Isolagen injected dermis (Fig. 8). The results of Chernoff's laser study further support the previously cited work of Rosenberg et al¹⁰ and Goldberg et al,⁷ as well as the authors' experience that a dermis that is rich in autologous fibroblasts responds to other modalities of therapy in a far superior fashion than a nontreated dermis that has a diminished population of collagen and fibroblasts.



Figure 9. Acne scarring on cheek before (A) and 3 1/2 years after (B) autologous cultured fibroblasts (Isolagen) injection.



Figure 10. Perioral, Marionette, and vermillion rhytids before (A) and 4 years after (B) autologous cultured fibroblasts (Isolagen) injection.

SUMMARY OF CLINICAL RESULTS AND COMBINATION THERAPY

In the authors' experience the use of autologous fibroblasts produces the most dramatic responses in people suffering from acne scarring (Fig. 9). It provides an opportunity to repopulate the dermis with fibroblasts and give fill as well as create a better bed for the use of lasers. Isolagen is not effective on ice-pick acne scars and does not change the thin shiny epithelium that is seen with acne scars that have healed by secondary intention. Isolagen can be very effective when used alone and can provide continuing long-term correction, which is most dramatic in the perioral rhytids (Fig. 10). Isolagen can help correct dermal defects that are often associated with the deficiencies of prominent nasolabial folds (Fig. 11). Isolagen can enhance the results of either erbium, CO₂, or the Nd:YAG lasers. A low-power, single-pass CO₂ laser or superficial Er:YAG (1 joule, three-pass treatment) can

be very effective when the area is pretreated with Isolagen. The lasered areas that document the superficial level of the laser treatment would epithelialize within 3 to 5 days. Laser therapy should only be expected to decrease the depth of rhytids by one Baker classification. The authors, however, have found that superficial lasering preceded by Isolagen has improved the depth of wrinkles by 2 to 3 Baker grades of severity (Fig. 12).

The use of Isolagen with bovine collagen has extended longevity of the bovine collagen for an indefinite period in many patients. At a minimum the authors expect bovine collagen to provide a dramatic early fill and to allow the Isolagen to provide a long lasting correction that would not have been obtained by using bovine collagen alone (Fig. 13). It is important not to inject the Isolagen and the bovine collagen at the same time, but rather to first inject the bovine collagen at two separate sessions 1 week apart, and then perform two Isolagen injections spaced 2 weeks from

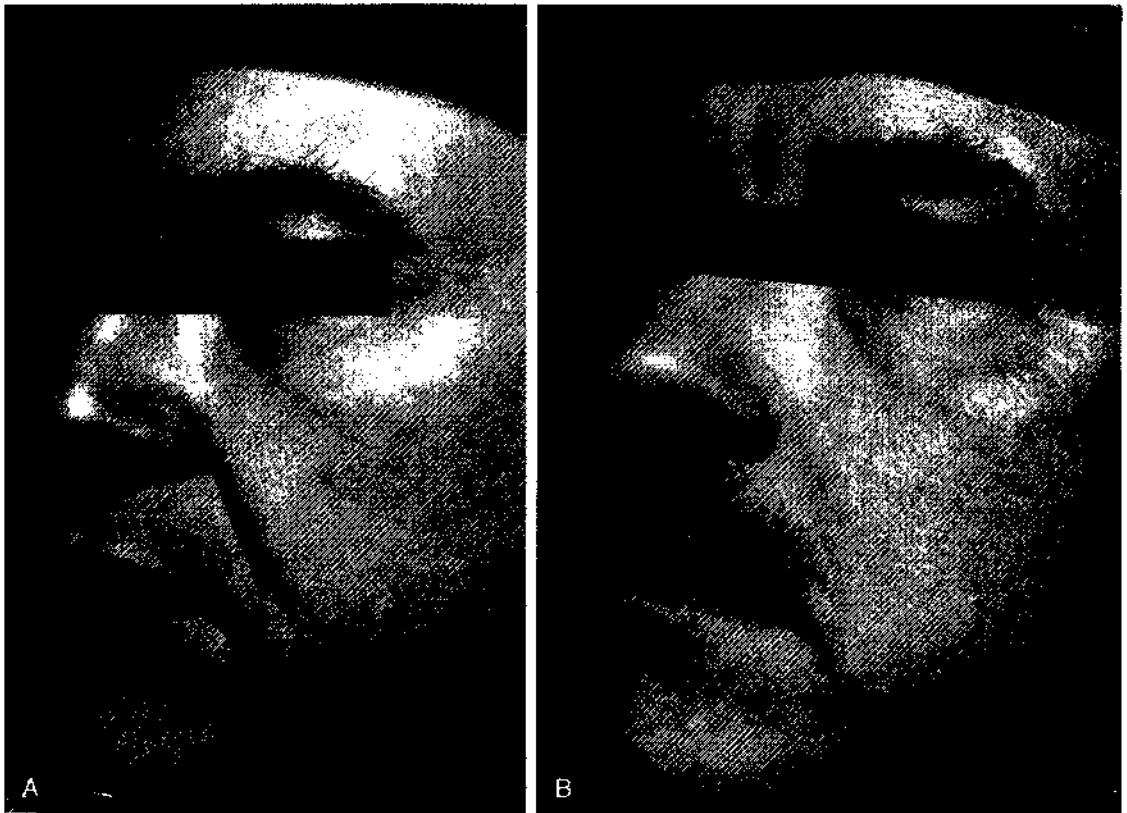


Figure 11. Prominent nasolabial folds before (A) and 2 years after (B) three autologous cultured fibroblasts (Isolagen) injections.



Figure 12. Perioral, marionette, and vermilion rhytids before (A) and 3 years after (B) three passes of 1J/cm² Er:YAG laser.



Figure 13. Prominent nasolabial folds before (A) and 18 months after (B) combined autologous cultured fibroblasts (Isolagen) and bovine collagen injection.



Figure 14. *A*, Glabellar scar following browlift with overresection of the glabellar muscles. *B*, Dramatic correction of dermis and subcutaneous layer; however, treatment of the epidermis and loss of deep muscle would further improve the area.



Figure 15. Saddle nose depression and nasolabial folds 30 years after rhinoplasty (*A*) and 30 months after correction with three autologous cultured fibroblasts (Isolagen) injections (*B*).

the bovine treatment and 2 weeks from each other. It has been the experience at the different test centers that Isolagen is most effective in the younger age group and for acne and post-traumatic scars (Figs. 14 and 15).

DISCUSSION

Of the 4800 injections, there have been 11 reports of reaction. The most severe of these reactions has been redness and induration for 10 days following treatment. There have been no reports of generalized allergic reaction or skin slough, nodule formation, keloid formation, or overcorrection. There have not been reports of reabsorption or loss of correction, and Isolagen is ultimately more cost-effective as the results are long-lasting and require fewer total treatments.

CONCLUSION

Isolagen has proven to be safe and effective in the multicenter studies that have been underway since 1995. It is a versatile product that can be used alone or in conjunction with laser resurfacing, chemical peels, bovine collagen, and other nonviable fillers. It appears that Isolagen represents the first step in autologous cellular treatment of scars and rhytids by treating the underlying cause of these conditions, which is the lack of collagen and fibroblasts in the affected areas of the dermis.

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